INTERFACIAL TENSION MEASUREMENTS IN AN OIL/WATER/BACTERIA SYSTEM BY LASER-LIGHT SCATTERING

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ABSTRACT

Microbial Improved Oil Recovery (MIOR) utilizes the growth of oil degrading bacteria on the oil/water interface to improve the effects of water flooding. Published results [1] show that stimulating bacterial growth in core samples can give significant increase in oil recovery. Reduction in interfacial tension (IFT) by bacterial action is one of the proposed mechanisms for this increased oil recovery. This type of bacteria will grow at the water-oil interface since they need nutrients from the water and components from the oil as a carbon source. To access the oil droplets from the water phase, the bacteria reduce the interfacial tension. IFT would generally have to be lowered by 3-4 orders of magnitude to explain the low residual oil saturations observed.

Measurements of interfacial tension in bacterial systems are difficult due to a growth period of several days in order to reach a sufficiently large bacterial population. This study has applied an advanced laser-light scattering technique to measure interfacial tension in an oil/water/bacteria system, where the duration of the experiments was not limited by the method. Interface laser-light scattering has allowed for IFT measurements over several orders of magnitude without disturbing the bacterial growth of the system. The oil degrading bacteria strain A14101 used in the present work reduced the oil/water IFT from 35 mN/m to 0.17 mN/m over a period of about 2 weeks, before stabilizing. No further reduction in IFT was measured, but it is not likely that this value is the lower limit for the bacterial system due to limitations in the experimental setup.

INTRODUCTION

The challenge to increase the oil recovery from the reservoirs is a driving force behind the efforts to come up with alternative and cost efficient recovery processes [2]. Consequently, various Improved Oil Recovery (IOR) methods are being evaluated including the use of microbial processes in the reservoirs. According to Brown et al. [3], MIOR was first introduced by Beckman in 1926. Claude ZoBell applied for a patent on the principle behind this method in 1944 [4], while research and field testing during only the last decade have demonstrated the industrial potential for exploitation of

microorganisms for IOR [5]. MIOR may be regarded as an assisted waterflood where bacterial growth is leading to mobilization and further production of remaining oil by extended waterflooding.

In spite of numerous investigations of the MIOR processes and the description of bacterial growth and the resulting production of metabolites, the mechanisms behind oil mobilization are still poorly understood. Several mechanisms are proposed to explain the IOR effects of bacterial growth in oil reservoirs, including reduced IFT and changed wettability [2]. In an extended MIOR core flood the residual oil was lowered down to about 3 % [1]. The oil production rate during the microbial waterflooding was approximately constant even at low oil saturation values. This is in agreement with a gradual lowering of the IFT, thereby releasing previously trapped oil. A combination of IFT reduction and other mechanisms is also possible, but more accurate measurements of IFT are necessary to help resolve this question.

IFT measurements for water-oil systems are usually performed by a conventional tensiometer, using the ring method, the pendant drop or the spinning drop methods. These are relatively fast methods, well suited for measurements on equilibrated fluids. These methods are not very well suited for long time measurements with gradually changing IFTs as are needed to monitor microbial processes. The pendant drop method used for similar bacterial systems [6] was not able to give stable measurements for prolonged times. With interface laser-light scattering, the microscopic fluctuations of the interface can be probed by a laser beam without significant perturbation of the system, and the IFTs can be measured in the range from 10^{2} mN/m down to 10^{-5} mN/m.

MICROBIOLOGY

The ability of microbes to grow on oil compounds (hydrocarbons) as sole carbon and energy source was once believed to be restricted to aerobic bacteria, but has within the last two decades also been recognized for anaerobic bacteria [7]. In this paper we will only address the aerobic hydrocarbon-degrading bacteria. Aerobic bacteria are a diverse group of bacteria defined by the fact that they use oxygen as electron acceptor in a respiration process. During aerobic degradation of both aliphatic and aromatic hydrocarbons, oxygen is also involved in the first activating step, which involves the membrane-bound enzyme oxygenase. Oxygen is obtained from the water phase, as are mineral nutrients (nitrates and phosphates), which are essential in order to maintain cellular activities and cell division. Since hydrocarbons have limited solubility in water, bacteria have to modify the interface environment in order to attain contact with both phases. The hydrocarbon-degrading bacteria overcome this obstacle by producing biosurfactants, which are a heterogeneous group of surface-active molecules with a hydrophilic and a hydrophobic moiety [8, 9]. There are two different strategies used by bacteria in biosurfactant production [9, 10, 11]. One of them is production of extracellular emulsifying agents, creating an oil/water emulsion. The other is production of cell-bound surfactants, which affect only the oil-water interface where active bacteria are located. The cells grow and divide at the interface and the result is a reduced oil-water IFT.

The latter strategy is expected to happen during MIOR [1]. Reduction in hydrocarbon-water IFT by biosurfactants is well documented [11, 12]. A reduction in IFT during bacterial growth at the interface has also been described [6], but the need for a more sensitive method is addressed in this study.

The ability to grow on hydrocarbons is widespread among bacteria found in both terrestrial and aquatic environments, including oil reservoirs. Bacteria in oil reservoirs may be indigenous or introduced through water-flooding [13]. As mentioned, bacteria may sustain growth on hydrocarbons under both aerobic and anaerobic conditions, the latter including sulfate- and nitrate respiration [14, 15, 16]. The aerobic MIOR process is investigated by laboratory experiments [1] and is now implemented at the Norne Field in the Norwegian Sea.

REDUCTION OF INTERFACIAL TENSION

The reduced IFT reported in field applications [17] and in many laboratory investigations [18] is believed to have an important effect on oil recovery by bacterial stimulation. In order to explain the very low residual oil saturation in some of the laboratory experiments, the IFT may need to be lowered by up to four orders of magnitude [18] in a water-wet system. Such low IFT values have not yet been reported for such systems.

As described earlier, some bacteria are known to produce surface-active components [19]. However, there are indications that the bacterial cell itself is important in this connection. Comparing experiments where only bacterial metabolites are injected, with experiments where both living bacteria and their metabolites are injected indicate this. It is shown that metabolites alone give less recovery effect than the combined use of bacteria and metabolites in a core flood [20].

PRINCIPLE OF INTERFACE LASER-LIGHT SCATTERING

IFT measurement by laser-light scattering takes advantage of microscopic interfacial roughness caused by random molecular motion in the adjacent fluid phases. The position-time-dependent roughness amplitude is very small – typically of the order of nanometres. The roughness can be considered as superimposed sinusoidal interfacial (capillary) waves of different wavelengths Λ. Formally, this is equivalent to expanding the roughness amplitude into a set of Fourier components, or modes, each of which can be described by (i) a wave number q ($= 2\pi/\Lambda$) and (ii) a q-dependent mode amplitude (which fluctuates due to the random nature of the interfacial perturbations).

When a fluid interface is illuminated by a laser beam, the interfacial roughness causes a small part of the incident light to be diffusely scattered out of the laser beam. Optically, each Fourier component of the roughness amplitude acts as a sinusoidal diffraction grating scattering light into a well-defined direction. Hence, a single interfacial mode of wave number q can in principle be probed by detecting light scattered under a well-defined angle θ. With the present experimental set-up ($\theta \lesssim 2°$, measured in a plane

perpendicular to the plane of incidence) the scattering angle θ and the corresponding interfacial mode wave number q are proportional [21]:

$$\theta = q / k \tag{1}$$

Here, k is the wave number of light, defined by $k = 2\pi / \lambda$, where λ is the wavelength. Interfacial modes probed in the present work had q-values in the range from approximately $3 \cdot 10^4$ m^{-1} to $4 \cdot 10^5$ m^{-1}, corresponding to wavelengths Λ in the range from 200 µm to 20 µm.

The intensity of light scattered under an angle θ has the same time dependence as the (fluctuating) amplitude of the interfacial mode of wave number q. It is this time dependence that is measured. The information contained in the fluctuating intensity is extracted by computing the degree of correlation between intensities observed at different points of time. Figure 1 shows two typical correlation functions acquired for the oil/water system studied in the present work – one acquired at the time bacteria were injected into the IFT measurement cell and another two weeks after bacteria injection, at which time the IFT had decreased by a factor of 200. These two correlation functions illustrate the sensitivity of the analysis to a decrease in IFT, which is reflected mainly in the decreasing oscillation frequency of the correlation function.

The method of data analysis, which has been discussed in detail elsewhere [22], involves the fitting of a theoretically computed correlation function to the experimental data. This expression is essentially the Fourier transform of the interfacial fluctuations' theoretical power spectrum [21], which includes a number of fluid parameters, notably IFT and fluid densities and viscosities.

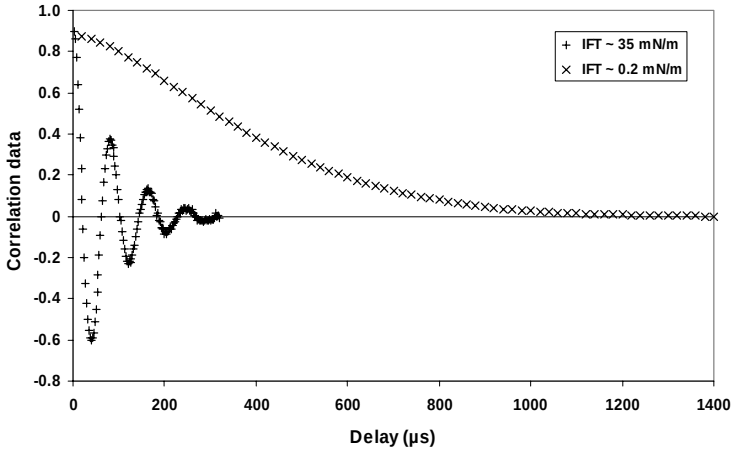

Figure 1 Typical correlation data (q ~ $7 \cdot 10^4$ m^{-1}) for oil/water system with bacteria: (+) at the time bacteria were injected into the IFT measurement cell, when the IFT was 35 mN/m, and (×) 14 days later when the IFT had decreased to ~ 0.2 mN/m.

The shape of the correlation function is determined by the balance between restoring and damping forces at the fluid interface. The principal restoring force is that of interfacial tension whereas the principal damping mechanism is due to the viscosity of the adjacent fluid phases. The balance between the restoring and damping forces is conveniently given by the dimensionless quantity Y [21]:

$$Y = \gamma(\rho_o + \rho_w)/[4(\eta_o + \eta_w)^2 q] \quad (2)$$

where, γ is the IFT; ρ_o and ρ_w are oil and water densities; and η_o and η_w are oil and water viscosities. For $Y \gtrsim 0.1$, as was mostly the case in the present measurements, the analysis allows simultaneous determination of the IFT and the viscosity sum. Fluid densities are required as input parameters of the analysis. It can be shown from the general theoretical expression for the correlation function that its frequency is in this case roughly proportional to $[\gamma/(\rho_o + \rho_w)]^{1/2} q^{3/2}$ [21]. The viscosity sum is reflected mainly in the temporal damping of the correlation function, which is roughly proportional to $2[(\eta_o + \eta_w)/(\rho_o + \rho_w)]q^2$ [21].

Given negligible uncertainty in the input density values, both the IFT and the viscosity sum can usually be determined to an accuracy better than 2-3 %. The method is particularly well suited for low IFT values (down to 10^{-5} mN/m) because the intensity of scattered light increases with decreasing IFT [21].

EXPERIMENTAL

Experimental setup

A schematic diagram of the optical system is shown in Figure 2. The laser was a 5 mW Uniphase 1125 He-Ne laser used in conjunction with a horizontally oriented polarizer (P). The beam was expanded, spatially filtered, and focused by the L_1-A_1-L_2 lens-aperture combination. A weak transmission diffraction grating (G) placed in the converging beam, provided reference beams (of which one is shown in Figure 2) at various small angles θ to the main beam. The reference beams' intensities were adjusted to a suitable level by use of an attenuator (ATT) and made to cross at the fluid interface by the lens L_3. One of the reference beams (which define the scattering angles) was then selected and passed through the A_2-L_4-A_3 lens-aperture combination to the photocathode of the photo multiplier tube (PMT) where it mixed with light scattered out of the much stronger main beam at the fluid interface. This optical mixing technique is essential for enhancement of the very weak signals in interface light scattering.

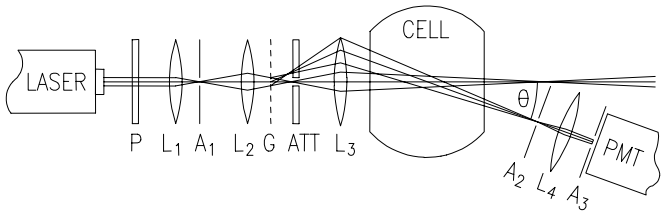

Figure 2 Schematic diagram of the optical set-up of the interface laser-light scattering spectrometer.

Fluids

The oil was n-dodecane (Merck, purity > 99%). The density and viscosity at 20.0 °C were 748.8 kg/m^3 and 1.499 mPa.s, respectively [23].

The water used in the experiment was based on the water reported by Myhr *et al*. [24], modified according to Table 1. Trace elements and vitamin solution used are described by Widdel *et al*. [25] and Pfennig [26], respectively. The composition is given in Table 1, and the nutrient water density and viscosity at 20.0 °C were measured to be 1018.1 kg/m^3 and 1.074 mPa.s, respectively

Table1 Composition of nutrient water.

Component	Amount per litre
Sodium chloride, NaCl	20 g
Sodium sulphate, Na_2SO_4	4 g
Magnesium chloride, $MgCl_2 \cdot 6H_20$	3 g
Potassium chloride, KCl	0.5 g
Calcium chloride, $CaCl_2 \cdot 2H_2O$	0.15 g
Potassium phosphate dihydrogen solution, 140 mg KH_2PO_4 per 100 ml water	1 ml
Ammonium chloride solution, 620 mg NH_4Cl per 100 ml water	1 ml
Resazurin sodium salt, 0.2 % (w/v) $C_{12}H_6NO_4Na$ in dH_2O	100 µl
Vitamin solution	5 ml
Sodium carbonate hydrogen, 1M $NaHCO_3$ solution	6 ml
Trace elements	1 ml

Bacterial system

The bacterium was isolated from an oil reservoir model column. Crude oil was the sole carbon source and the system was flooded with a synthetic seawater medium [24]. The system was inoculated with a mixture of hydrocarbon-degrading bacteria enriched from oil installations in the North Sea, and had been running for approximately 9 months when aerobic hydrocarbon-degrading bacteria were isolated from the water face by dilution and subsequent plating on synthetic seawater agar [12]. The agar plates were incubated under aerobic conditions under crude oil vapor at room temperature. Colonies were collected after 5 and 14 days. The organism used in this study was designated strain A14101. Phylogenetic analysis showed that A14101 belong to the genus *Dietzia*, and the closest

validated related species is *D. maris*, formerly *Rhodococcus maris*, with 16S rRNA gene-similarity value of 99 %. Based on morphology, A14101 is similar to *D. maris*, with coccoid cells germinating into short rods [27]. Orange colonies were observed during growth of A14101 on agar and crude oil vapor. Growth of A14101 was also observed on *n*-dodecane.

Preparations
Prior to measurement of the IFT between dodecane and distilled water the cell was evacuated to a pressure smaller than 0.05 mbar and dry heat sterilized by heating to 150 °C. The cell was kept at this temperature for 4 hours before cooling to room temperature. Tubing and fittings that could not be sterilized this way were either boiled in distilled water for 10 minutes, flushed out with acetone, or scorched with a gas burner.

Although full sterilization could not be achieved for all equipment involved, it is reckoned that the above precautions were sufficient to reduce spurious microbial activity to an acceptable level. The cell was charged with approximately 40 ml dodecane and 28 ml nutrient water. The nutrient water was passed through a 0.22 µm filter before being charged to the cell. This was done to remove dust particles that could otherwise have adsorbed to the oil/water interface and perturbed the light scattering measurements.

Measurement procedures
The IFT was measured at intervals over a period of two days until an equilibrium IFT value was established. Having established the reference IFT value for the pure liquid system, bacteria were introduced into the water phase. Approximately 6 ml bacteria suspension followed by 3 ml pure nutrient water (to flush out the injection tubing) was sucked into the sample chamber by expanding the cell volume. The vertical level of the oil/water interface was adjusted and the IFT was measured at intervals over a period of several weeks. As the IFT decreased (as a result of microbial activity) the range of scattering angles was shifted to smaller values – typically 0.2° to 0.5°. The rationale for this was twofold:

1. Because the scattered intensity from capillary waves increases with decreasing wave number q (corresponding to decreasing scattering angle), adequate signal-to-noise ratio could be maintained despite increasing stray light level due to increasing bacteria concentration at the fluid interface.
2. By probing smaller capillary wave numbers q it was ensured that the dimensionless quantity Y (Equation 2) remained larger than 0.1 for at least some of the probed q values, thus allowing simultaneous determination of the IFT and the oil/water viscosity sum.

At the end of the test, water was sampled from the light scattering cell for determination of bacteria density.

RESULTS

Dodecane/nutrient-water system

Figure 3 shows the IFT between dodecane and nutrient water at 20 °C. The main purpose of these measurements was to establish a constant reference IFT value for the system before introducing the bacteria. The data indicate that the system was probably not at equilibrium when the first measurement was taken at 2.4 hours. The subsequent data points yield the average IFT value 35.4 mN/m. The most likely cause for the relatively low equilibrium IFT value would seem to be surface active components in the nutrient water. Water used for the measurements was poured from a newly opened bottle, which was only lightly agitated before pouring. Hence, water was sampled from the top layer and it is possible that this water may have contained excess surface active components. This theory was strengthened by auxiliary IFT measurements by ring tensiometer. The exact equilibrium value was however not very important for the bacterial results, but rather a confirmation that the equipment and calculations were calibrated. Some of the aging effect could also be caused by small amounts of impurities in the oil phase.

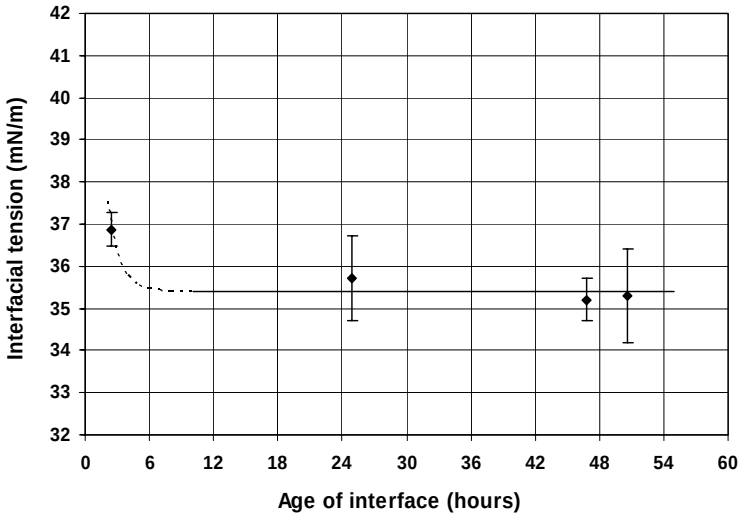

Figure 3 Light scattering measurements of IFT between dodecane and nutrient water at 20 °C versus age of interface. Solid line represents average of data points taken after equilibration of the system. Error bars indicate one standard deviation.

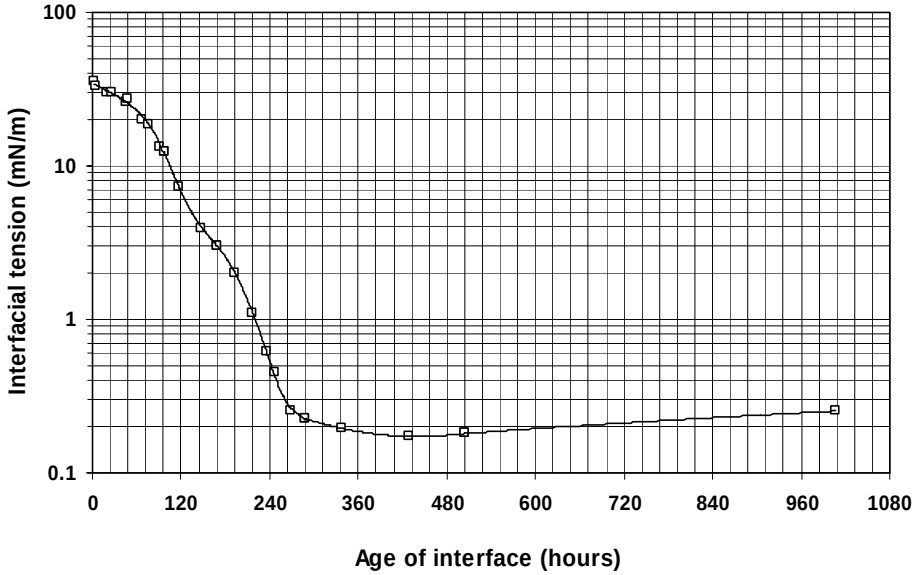

Figure 4 Light scattering measurements of IFT between dodecane and nutrient water with suspended bacteria (strain A14101) at 20 °C versus age of interface. Solid line is drawn within the uncertainty of the individual points (typically smaller than the plotted points) as a visual aid.

Dodecane/nutrient-water/bacteria system

Figure 4 shows measurements of the IFT between dodecane and nutrient water with suspended bacteria (strain A14101) at 20 °C. Zero age of interface refers to the time at which bacteria were introduced into the light scattering cell. The solid line is drawn within the uncertainty of the individual points (typically ~ 2-3 %, which is smaller than the plotted points) and is intended as a visual aid.

DISCUSSION

From Figure 4 it can be observed that although the initial rate of IFT decrease was relatively small, the rate increased significantly (in a logarithmic plot) after 3-4 days. Eighteen days after start the IFT had decreased by approximately two orders of magnitude, reaching a minimum value 0.17 mN/m. After this time there was no marked change in the IFT (excepting a slight increase over the next 2-3 weeks). This increase could be caused by the bacterial growth terminating and the effect on IFT slowly disappearing. An estimate of the amount of oxygen originally in place and a typical consumption rate of oxygen by the bacterial growth, supports this theory.

Towards the end of the experiment, visual inspection revealed a greyish film at the interface interspersed with darker grey areas (“islands”), which were probably bacteria colonies. These regions were sparsely distributed over the fluid interface – in total they covered no more than a few percent of the interfacial area. At the time the experiment

was terminated, the interface was seen to have 10-12 darker areas with diameters from below 1 mm up to 2-3 mm. The final bacteria density of water sampled from the system at the end of the experiment was found to be $8.8 \cdot 10^6$ cells per ml.

Although the illuminated area at the fluid interface (~ 15-20 mm^2) was restricted to 3-4 % of the total interface area, it was nevertheless much larger than the sparsely distributed interfacial inhomogeneities. Hence, the interfacial waves probed were not confined to any local inhomogeneity, which means that the deduced IFT value was probably representative of the overall interfacial tension. There was no indication of local variations of IFT within the probed interface area. Any such variation would give rise to local variations in interfacial wave frequencies. The temporal damping of the correlation function is very sensitive to superimposed signals of different frequencies. Hence, local IFT variation would result in anomalously large damping of the measured correlation function. The viscosity sum, which is deduced mainly from the temporal damping gave no indication of such anomalous damping. The scatter in the viscosity data is limited to approximately ± 4 %, which is no more than can be explained by statistical uncertainty due to relatively low signal strength (especially the first 3-4 days before the IFT had decreased significantly) and high stray light levels (caused by increasing bacteria concentration at the fluid interface). Thus, although increasing bacteria concentration at the fluid interface introduced local interfacial inhomogeneities, this is not believed to have resulted in biased IFT measurements.

Høiland [6] measured IFT on the same system with the same bacteria, and was able to record values down to only 10 mN/m with the pendant drop method. The main advantage of the light scattering method is that the IFT is determined directly from its effect on microscopic interfacial dynamics whereas in classical methods the IFT is deduced from macroscopic interfacial statics. In the pendant drop method, which is the only classical method with a suitable measurement range, the IFT is deduced from the shape of a liquid drop. The drop is suspended from a needle tip under the influence of gravity. To monitor the relatively slow IFT related processes in an oil/water/bacteria system it is required to keep the drop suspended at the needle tip for an extended period of time. As the drop may detach from the needle tip due to altered wetting conditions or decrease in IFT, this represents a challenge in itself. The light scattering method offers a more appropriate technique for IFT measurement in oil/water/bacteria systems.

CONCLUSIONS

- Interface light scattering has successfully been applied to monitor IFT decrease in a model oil/water system of dodecane and nutrient water, induced by bacterial activity at the oil/water interface.
- The oil/water IFT was reduced from 35 mN/m to 0.17 mN/m (a factor of approximately 200), over a period of two weeks. The observed microbial IFT-lowering effect is by far the largest reported hitherto in such a fluid system.
- The IFT decrease may have been limited to this value due to depletion of oxygen or some essential nutrients in the water causing the growth of bacteria to cease. Hence, there may be a potential for even larger IFT-decrease than the factor 200 established in this work.
- The light scattering method has distinct advantages over classical IFT measurement methods for MIOR for several reasons; IFTs must be monitored for long periods of time without disturbing the interface, and the range of IFT to be measured spans several orders of magnitude.

NOMENCLATURE

θ	Scattering angle
q	Wave number, m^{-1}
k	Wave number of light, m^{-1}
λ	Wave length of light, m
Λ	Wave length, m
γ	Interfacial tension, N/m
ρ_o	Oil density, kg/m^3
ρ_w	Water density, kg/m^3
η_o	Oil viscosity, Pa.s
η_w	Water viscosity, Pa.s

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